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PRINCIPAL INVESTIGATOR: Roger A. Greenberg

CONTRACTING ORGANIZATION: University of Pennsylvania
Philadelphia, PA 19104

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Roger A. Greenberg

E-Mail: rogergr@mail.med.upenn.edu

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Understanding molecular pathways that control resistance to chemotherapy/radiation and regulate metastasis are critical to improving breast cancer survival. We have been characterizing the biology behind gene signatures that either promotes DNA damage resistance or metastasis. Interestingly, these gene signatures are often populated with interferon-stimulated genes or pro-inflammatory mediators that respond to viral infection. This raises the question of what is behind the perplexing relationship between anti-viral responses and breast cancer progression. For this, we are focusing on the potential role of transposable elements (TEs), which are small pieces of DNA that have the ability to move within the genome and are often referred to as "jumping genes". Interestingly, expression of genes belonging to DNA damage resistance gene signatures are also induced after DNA damage and expression often associates with aberrant TE expression. These data raise the intriguing possibility that various pattern-recognition receptors (PRRs) that normally sense viral and bacteria nucleic acids and signal to interferon and pro-inflammatory pathways may provide a link between TE de-repression and expression of resistance and metastasis genes. Thus, the purpose of our Collaborative Idea Award proposal is to begin to explore the mechanisms of TE de-repression by DNA damage responses, how aberrant TEs are sensed, and the consequences of this on resistance

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## I. INTRODUCTION

Two-thirds of patients with micro-metastasis have cancer that is resistant to chemotherapy and one-third of patients with residual disease after surgery have cancer that is resistant to radiation. Thus, understanding molecular pathways that control resistance to chemotherapy/radiation and regulate metastasis are critical to improving breast cancer survival. Toward this end, we have been characterizing the biology behind gene signatures that either promotes DNA damage resistance or metastasis. Interestingly, these gene signatures are often populated with interferon-stimulated genes or pro-inflammatory mediators that respond to viral infection. This raises the question of what is behind the perplexing relationship between anti-viral responses and breast cancer progression. For this, we are focusing on the potential role of transposable elements (TEs), which are small pieces of DNA that have the ability to move within the genome and are often referred to as "jumping genes". Although TEs are normally repressed, recent sequencing of cancer genomes has revealed aberrant expression of TEs, suggesting that failure to properly silence TEs may be an important property that drives cancer-associated pathways. We have recently reported the phenomenon of Double Strand Break Induced Silencing in Cis (DISC), a process that relies on ATM and histone H2A ubiquitylation to silence transcription. Preliminary data suggest that the mechanisms involved in DISC are shared with cellular responses to restore TE repression after DNA damage, and these mechanisms may be diminished in cancer and result in aberrant TE expression. Interestingly, expression genes belonging to DNA damage resistance gene signatures are also induced after DNA damage and expression often associates with aberrant TE expression. These data raise the intriguing possibility that various pattern-recognition receptors (PRRs) that normally sense viral and bacteria nucleic acids and signal to interferon and pro-inflammatory pathways may provide a link between TE de-repression and expression of resistance and metastasis genes. Thus, the purpose of our Collaborative Idea Award proposal is to begin to explore the mechanisms of TE de-repression by DNA damage responses, how aberrant TEs are sensed, and the consequences of this on pathways involved in resistance and metastasis.

## II. BODY

Over the 2-year period encompassing this Collaborative Idea Award, we have made good progress on various parts of both Task 1 and Task 2 as outlined in the Statement of Work (SOW). Dr. Roger Greenberg has led task 1 and Dr. Andy Minn has led Task 2. For Task 1, we have initiated work on both Subtasks. For Task 2, we have initiated work on three out of the four Subtasks. Overall, our findings are novel and encouraging and have resulted in multiple high profile publications that significantly address the objectives of the proposed research. This progress is detailed below.

### IIA. Investigate mechanisms of DNA damage induced TE/a-satellite derepression in breast epithelial cells.

**Subtask 1.** Investigate ATM, Ubiquitin, and SUMO pathways of TE silencing after DNA damage in primary mammary epithelial lines and in breast cancer cell lines.

The primary objective of subtask 1 was to identify ATM dependent mechanisms of DSB silencing and relate these findings to the maintenance of genome integrity. To this end, we have identified SUMOylation as a critical component of silencing chromatin in cis to DSBs and to the maintenance of chromatin architecture at the DSB site. We have also established that persistent etoposide induced breaks silence transcription. These findings are currently being prepared as a manuscript (Harding, Greenberg et al in preparation).

**Subtask 2.** Develop a novel siRNA screen for genetic requirements for DSB induced TE reactivation.

The primary objective for Task 1 was to understand how DNA damage induces derepression of TEs. We have previously observed an ATM dependent suppression of local transcription in cis to the site of DNA double-strand breaks (DSBs). Similar mechanisms of DSB silencing were involved in suppression of TEs following DNA damage, inspiring the hypothesis that transfer of modifications that normally silence TEs to sites of DNA damage would lead to TE derepression and subsequent biological responses mediated by pattern recognition receptors. We have made considerable progress on both Subtasks by (i) delineating DSB silencing pathways at endogenous genomic locations, (ii) linking DSB silencing to TE derepression, (iii) developing siRNA screening technologies and acquisition of larger siRNA libraries, and (iv) documenting pattern recognition receptor involvement in interferon responsive gene expression following DNA damage. These are described below.

IIA1. To determine if DSB silencing pathways are repress transcription in cis to endogenous DNA damage sites (Subtask 1). To determine if DSB silencing mechanisms extend to endogenous genomic locations in

addition to reporter systems, we developed an inducible nuclease system that could create persistent DSBs in either the rDNA based on the prior work of the Kastan group with the IPpoI nuclease (Berkovich et al. Nat Cell Biol 2007). In addition, we monitored nascent RNA synthesis overlying spontaneous DNA damage sites. Our findings are concordant in both systems. ATM was required for DSB induced silencing at rDNA breaks and at other spontaneous breaks in the nucleus, including at androgen receptor induced genes that had been silenced by etoposide, thus establishing the generality of DSB silencing mechanisms.

IIA2. Determine if DSB silencing mechanisms are engaged in repressing TE expression (Subtask 1). We have documented that expression of the lytic phase HSV-1 gene ICP0 can reverse DSB silencing. Similar results were obtained using Gam1, an adenoviral protein that inhibits SUMO E1 ligase function, reinforcing the concept that SUMOylation is important for DSB silencing. We now link these observations to DNA damage induced TE repression. Expression of ICP0 or Gam1 strongly enhanced TE and a-satellite expression following etoposide treatment as did ATM inhibition. These findings establish the shared mechanisms of transcriptional repression between DSB silencing and TE repression.

IIA3. Develop siRNA screening technologies to identify genes involved in TE repression (Subtask 2). We have not yet performed the siRNA screen to identify additional genes involved in DSB silencing. This was not accomplished due to the technical difficulty of detecting TEs in a high content imaging analysis format. We have decided not to pursue this approach given the technical issues and because of the progress we have made regarding other aspects of the proposal.

IIA4. Determine if pattern recognition receptors mediate interferon responsive gene expression following DNA damage (Subtask 2). A central question of this proposal is whether TE activation of pattern recognition receptors following DNA damage will induce the IRDS. This would necessitate that one or more pattern recognition receptors would be required for interferon responsive gene expression following DNA damage. We have now established this to be the case. Rig-I null mouse cells did not increase interferon responsive gene expression following ionizing radiation or oncogenic Ras expression. Moreover, reconstitution with a wildtype Rig-I allele, but not a RNA binding domain mutant restored interferon responsive gene expression. These findings establish an important link between endogenous RNA recognition by Rig-I and DNA damage dependent interferon responsive gene expression that may underlie the IRDS.

**IIB) Task 2. Examine how derepression of TEs signal to STAT1/IRDS and pro-inflammatory genes to influence breast cancer.**

**Subtask 1.** Investigate if TE expression induces the IRDS and influences DNA damage resistance.
**Subtask 2.** Investigate whether STAT1 regulates TE or components of the TE silencing machinery.
**Subtask 3.** Determine if TE expression influences breast cancer invasion and metastasis.
**Subtask 4.** Examine if IRDS-associated intracellular nucleic acid sensors recognize de-repressed TEs and whether this regulates the effects of TE expression.

A central concept in our hypothesis for how de-repressed TEs might drive breast cancer progression is that they can engage PRRs to activate distinct signaling events important for resistance and/or metastasis. In our original proposal, we presented preliminary data that knockdown of several PRRs could decrease both invasion and metastasis gene expression. In the first year of this award, we elected to focus efforts on establishing that PRRs can influence metastasis (Subtask 4). In so doing, we have gained significant insight. This insight has also informed the direction and details of the other subtasks. Thus, we describe below our progress as it primarily relates to Subtask 4 but also describe progress for Subtask 1 and 3, both of which have been influenced by our preliminary findings.

IIB1. Pattern recognition receptors regulate breast cancer metastasis to the lung (Subtask 4). Pattern recognition receptors (PRRs) represent several families of receptors that normally recognize bacterial or viral pathogen motifs. Given our interest in how TEs might engage PRRs to signal, we were particularly interested in RIG-like receptors (RLRs), IFI16 family receptors, and several Toll-like receptors (TLRs), which are all known to recognize pathogenic RNA and DNA. To drill-down on particular candidate PRRs, we performed computational predictions using primary human breast cancer expression data to identify PRRs that best predict metastasis gene signature expression. For the metastasis gene signature, we used our previously describe lung metastasis gene signature (LMS), which experimentally regulates and clinically predicts breast cancer metastasis selectively to the lung. Computational analyses revealed that PRRs significantly predictive of LMS expression in primary human tumors include: several TLRs, AIM2, IFI16, and RIG-I (Figure XA).

In order to test whether any of the predicted PRRs might functionally control lung metastasis, we used shRNA gene targeting and the LMS(+) 4175 lung metastatic cell line derived from MDA-MB-231 breast cancer cells. To target the TLRs, we knocked down MYD88, which is a common adaptor protein important for downstream signaling. IFI16 and RIG-I were directly targeted with shRNAs to each. These engineered 4175 cells were then tested in a lung metastasis assay using immunocompromised mice. Non-invasive bioluminescence imaging revealed that knockdown of IFI16 and RIG-I significantly inhibited metastasis (Figure XB) without having a general effect on cell growth in vitro or on growth as a subcutaneous tumor (Figure XC). MYD88 also potently inhibited metastasis; however, we opted to focus on IFI16 and RIG-I since several TLRs could explain the MYD88 effect. Inspection of the lungs from mice harboring RIG-I and IFI16 knockdown cells revealed a large number of breast cancer cells spread throughout the lung parenchyma but few large outgrowths compared to the control tumors. These histological findings suggest a defect in macrocolonization. In total, these data demonstrate that PRRs can regulate breast cancer metastasis to the lung.

IIB2. Pattern recognition receptors control metastasis gene programs preferentially in vivo rather than in vitro (Subtask 4). In the 4175 breast cancer cells, several genes in the LMS control lung metastasis by regulating various steps in the metastatic cascade. To examine if the ability of IFI16 and RIG-I to control lung metastasis is correlated to changes in LMS gene expression, we examined a panel of LMS genes by qRT-PCR. Surprisingly, most LMS genes only showed a marginal decrement in expression when either RIG-I or IFI16 were inhibited and genes were examined in tissue culture. However, similar analyses using RNA extracted from in vivo tumors demonstrated a more dramatic decrease in several LMS genes. Thus, these observations suggest that cells of the lung microenvironment may contribute to the ability of PRRs to regulate LMS genes.

IIB3. Exosomes produced by stroma fibroblasts influence metastasis genes in a PRR-dependent manner (Subtask 4). Since lung fibroblasts are the most abundant cell in the lung parenchyma, we investigated whether these stroma cells might be involved in regulating LMS genes. To begin, conditioned media (CM) from mouse fibroblasts (L-cells) was applied to 4175 cells. This resulted in the upregulation of several LMS genes. This induction by CM was PRR-dependent. Thus, these data suggest that a secreted factor(s) from mouse fibroblasts can activate PRRs to induce LMS genes.

Typically, IFI16 and RIG-I engage viral DNA and/or RNA after viruses gain entry into a cell through membrane fusion or endosomes. Recent evidence suggests that some PRRs can be activated by exosomes, which are small membrane vesicles derived from multivesicular bodies. In fact, exosomes have been shown to play a role in promoting metastasis. Therefore, to investigate if exosomes might contribute to the secreted activity present in CM from mouse fibroblasts, we depleted and purified exosomes from CM. When added to 4175 cells, the exosome-depleted CM had reduced activity in stimulating LMS genes, while the purified exosomes could induce LMS gene expression. In total, these data suggest that exosomes in the CM from fibroblasts can stimulate LMS gene in a PRR-dependent manner.

IIB4. Exosomes are enriched in transposable elements (Subtask 3). Since RIG-I is known to bind to RNA and IFI16 binds to DNA, we reasoned that if exosomes stimulate LMS genes through RIG-I and/or IFI16, the activating ligand in the exosomes is likely DNA and/or RNA. Since our hypothesis is that TEs engage PRRs to promote metastasis, we began exploring whether TEs might be contained in exosomes and serve as candidate ligands for PRRs. For this, RNA from exosomes (exoRNA) was purified and examined for TE expression. Preliminary results indicate that exoRNA is indeed enriched in several TEs compared to cellular RNA. This enrichment over cellular RNA was not observed with GAPDH and 18S rRNA. We are currently performing unbiased exoRNA sequencing to further characterize these results. Thus, exoRNA may be enriched in TEs, suggesting that TEs might be a candidate ligand for PRR activation by exosomes.

IIB5. Transposable elements activate RIG-I signaling (Subtask 1). To investigate whether TEs can functionally serve as ligands to activate PRRs, we first introduced TEs into cells by standard DNA transfection. As a read-out for RIG-I activation, we monitored expression of the RIG-I target gene interferon-beta. To assess the specificity of these results, several unrelated mRNAs were also tested. This assay demonstrated that several TEs could induce IFN-beta in a RIG-I-dependent manner. In contrast, unrelated mRNAs had a much smaller effect. As an alternative and more direct approach, we purified in vitro transcribed TE RNA and packaged it into synthetic lipid vesicles to mimic exosomes. The ability of these TE RNAs to activate RIG-I was again determined by examining interferon-beta gene expression. As a positive control, we used a HCV RNA previously characterized to activate RIG-I. Negative controls include a mutant of HCV RNA devoid of RIG-I activating properties and several unrelated mRNAs. Preliminary results demonstrate that HCV RNA potently induces IFN-beta gene expression in a RIG-I-dependent manner, while mutant HCV RNA does not.

Interestingly, Alu RNA is also able to induce IFN-beta gene expression in a RIG-I-dependent manner. In total, these preliminary data suggest that TEs can activate RIG-I and are candidate ligands contained in exosomes that may activate PRRs.

IIB6. Ability of transposable elements to directly engage RIG-I (Subtask 4). RIG-I is known to bind to viral RNA and recent structure/function studies have elucidated several RNA properties that are important for binding. Some of these properties are potentially shared with TEs, including lack of a 5' cap and RNA size. To directly test whether TEs can bind to RIG-I, we initiated a collaboration with Dr. Joseph Marcotrigiano to test whether Alu RNA can bind to recombinant RIG-I. Preliminary results are thus far inconclusive. The positive control HCV RNA has demonstrated binding activity in a gel shift assay, while the HCV mutant RNA fails to bind. Binding by Alu is indeterminate. We are currently optimizing assay conditions.

As a complementary approach to the in vitro binding assay, we are also optimizing in vivo binding methods. For this, we have conducted pilot experiments to immunoprecipitate RIG-I to assess binding by TEs. Several technical steps have been attempted, including cross-linking, sonication conditions, examination of endogenous versus exogenous ligands, etc. As comparison, positive controls include Sindai virus (SeV) RNA, which has previously been shown to bind to RIG-I using similar immunoprecipitation methods. To-date, we have successfully demonstrated that SeV infection results in SeV RNA binding to RIG-I preferentially over IFN-beta or RIG-I mRNA. Similar experiments using transfected TE revealed RIG-I, but not RIG-I mutated in its RNA binding domain, binds to TE RNA. Although further optimization is needed, we anticipate that these methods will allow us to test TE binding in vivo and perform unbiased sequencing of the RIG-I-bound RNA. Our goal will be to use these assays to interrogate if TEs in exosomes can bind to RIG-I.

IIB7. Interpretation, limitations, and future directions. To-date, our data demonstrate that PRRs, in particular RIG-I and IFI16, can regulate breast cancer lung metastasis and lung metastasis gene expression. Regulation by PRRs appears to be linked to the tumor microenvironment as changes in LMS genes are markedly more pronounced in vivo compared to in vitro. Consistent with this, exosomes derived from mouse fibroblasts appear to induce several LMS genes and may do so in a RIG-I-dependent manner. Direct examination of RNA from TEs reveals enrichment of several TEs. When introduced into cells, these TEs can activate gene expression by RIG-I. Accordingly, preliminary results suggest that TEs can bind to RIG-I in vivo. In total, these data point toward TEs from exosomes engaging PRRs to regulate lung metastasis.

By opting to focus on Subtask 4 prior to the other subtasks, this has provided potentially important insight into the direction and interpretation of the other subtasks. Our original hypothesis posits that breast cancer intrinsic TEs bind to RIG-I. If exosomes from the microenvironment primarily drive metastasis by PRRs and if TEs contained in exosomes can serve as ligand for PRRs, this could suggest that TEs that originate from the stroma engage RIG-I. Such potential findings are not incompatible with our original hypothesis. Recent studies demonstrate that some breast cancer cells that include MDA-MB-231 cells are poor producers of exosomes. To circumvent this defect in exosome production, exosomes from neighboring cells can be taken up, used to package breast cancer proteins, and secreted in exosomes to make it available for the breast cancer to use. Similarly, we envision that breast cancer TEs may be similarly routed using exosome from fibroblasts.

There are some current obstacles and limitations that we are addressing. First, although TE binding to RIG-I is suggested, the specificity of these findings is uncertain. To address this, we are currently performing unbiased sequencing of RIG-I-bound RNA. We anticipate that these sequencing results will also inform us on the complexity of the TE landscape that might regulate metastasis. From this, we plan to select TEs that are enriched in RIG-I binding and test them for their ability to influence breast cancer progression as described in Subtasks 1 and 3.

## III. KEY RESEARCH ACCOMPLISHMENTS

## IV. REPORTABLE OUTCOMES

*IVA. Publications*

Minn

1. Boelens MC, Wu TJ, Nabet BY, Xu B, Qiu Y, Yoon T, Azzam DJ, Twyman-Saint Victor C, Wiemann BZ, Ishwaran H, Ter Brugge PJ, Jonkers J, Slingerland J, **Minn AJ**. Exosome transfer from stroma to breast

cancer cells regulates therapy resistance pathways. *Cell*. 2014 Oct 23;159(3):499-513.

2. Mucaj V, Lee SS, Skuli N, Giannoukos DN, Qiu B, Eisinger-Mathason TS, Nakazawa MS, Shay JE, Gopal PP, Venneti S, Lal P, **Minn AJ**, Simon MC, Mathew LK. MicroRNA-124 expression counteracts pro-survival stress responses in glioblastoma. *Oncogene*. 2014 Jun 23. doi: 10.1038/onc.2014.168. [Epub ahead of print]

3. Mathew LK, Skuli N, Mucaj V, Lee SS, Zinn PO, Sathyan P, Imtiyaz HZ, Zhang Z, Davuluri RV, Rao S, Venneti S, Lal P, Lathia JD, Rich JN, Keith B, **Minn AJ**, Simon MC. miR-218 opposes a critical RTK-HIF pathway in mesenchymal glioblastoma. Proc Natl Acad Sci U S A. 2014 Jan 7;111(1):291-6.

Greenberg

1. Solyom S, Aressy B, Pylkäs K, Patterson-Fortin J, Hartikainen JM, Kallioniemi A, Kauppila S, Nikkilä J, Kosma VM, Mannermaa A, **Greenberg RA***, Winqvist R* Recurrent breast cancer predisposition-associated *Abraxas* mutation disrupts nuclear localization and DNA damage response functions of BRCA1. Science Trans Med 22;4(122):122ra23, 2012. Notes: *co-corresponding authors. Comment in: "Abraxas is a Breast Cancer Susceptibility Gene". *Cancer Discovery* April 01, 2012 vol. 2 no. 4 p. 296296 2012.; NCI Cancer Bulletin (http://www.cancer.gov/ncicancerbulletin/030612/page3#d).

2. Beishline K, Kelly CM, Olofsson BA, Koduri S, Emrich J, **Greenberg RA**, Azizkhan-Clifford J. SP1 facilitates DNA double-strand break repair through a nontranscriptional mechanism. Mol Cell Biol. 32(18): 3790-9 2012.

3. Guzzo CM, Berndsen C, Zhu J, Gupta V, Datta A, **Greenberg RA**, Wolberger C, and Matunis MJ. RNF4-Dependent Hybrid SUMO-Ubiquitin Chains are Signals for RAP80/BRCA1 Recruitment to Sites of DNA Damage. Science Signaling 5(253):ra88, 2012.

4. Domchek SM*, Tang J, Jill Stopfer, Lilli DR, Tischkowitz M, , Monteiro ANA, Messick TE, Powers J, Yonker A, Couch FJ, Goldgar D, Nathanson KL, Foulkes WD **Greenberg RA***:Biallelic deleterious *BRCA1* mutations in a woman with early-onset ovarian cancer. Cancer Discovery 3: 399-405 2013. Epub ahead of print. Notes: *co-corresponding authors. *Highlighted in Cancer Discovery 2013: D'Andrea AD. BRCA1: A Missing Link in the Fanconi/BRCA Pathway.*

5. Tang J, Cho NW, Cui G, Manion EM, Shanbhag NM, Botuyan MV, Mer G, **Greenberg RA**: Acetylation limits 53BP1 accumulation at DNA double-strand breaks to promote homologous recombination. Nat Struct Mol Biol *20: 317-25, 2013. Notes: Highlighted in Nature Reviews Mol Cell Biol 2013: Du Toit A. DNA damage: Limiting 53BP1.*

6. Zheng H#, Gupta V#, Patterson-Fortin J#, Bhattacharya S#, Katlinski K, Wu J, Varghese B, Carbone CJ, Aressy B, Fuchs SY*, Greenberg RA*: A BRISC-SHMT Complex Deubiquitinates IFNAR1 and Regulates Interferon Responses. Cell Reports [Epub ahead of print], 2013 Notes: # co-first author* co-corresponding author.

7. Cho NW, Dilley RL, Lampson MA, Greenberg RA: Interchromosomal Homology Searches Drive Directional ALT Telomere Movement and Synapsis. Cell 159(1): 108-121, October 2014. Highlighted in Cell 2014, Arnoult N and Karlseder J. ALT Telomeres Borrow from Meiosis to Get Moving.

8. Sawyer SL, Tian L, Kähkönen M, Schwartzentruber J, Kircher M, University of Washington Centre for Mendelian Genomics, FORGE Canada Consortium, Majewski J, Dyment DA, Innes AM, Boycott KM, Moreau LA, Moilanen JS, Greenberg RA: Biallelic Mutations in BRCA1 Cause a New Fanconi Anemia Subtype. Cancer Discov 2014 in press.

*IVB. Abstracts*

Minn

1. Barzin Y. Nabet, Tony J. Wu, Mirjam C. Boelens, Brianne Z. Wiemann, Bihui Xu, and Andy J. Minn: Stroma-derived exosomes induce interferon-stimulated genes controlling breast cancer DNA damage resistance. AACR Advanced in Breast Cancer Research, San Diego, CA, October 2013.

2. Tony J. Wu, Barzin Y. Nabet, Mirjam C. Boelens, Bihui Xu, Taewon Yoon, Christina A. Twyman, and Andy J. Minn: Inhibition of NOTCH activation reverses tumor-stroma protection against radiation and chemotherapy. AACR Advanced in Breast Cancer Research, San Diego, CA, October 2013.

*IVC. Outside invited oral presentations*

Minn

1. "Emerging Approaches in Oncology: A Brainstorming ThinkTank", Banbury Center of Cold Spring Harbor Laboratory, Long Island, New York, June 2012.

2. "The Role of Inflammation and Immunity in Breast Cancer Etiology and Treatment", The Expedition Inspiration Fund for Breast Cancer Research, Sun Valley, Idaho, February 2013.

Greenberg

Mar, 2012 "Chromatin Responses to DNA Damage" Washington University of St. Louis School of Medicine, St. Louis, MO.

April 2012 "Chromatin Responses and Genome Integrity", Abcam Maintenance of Genome Stability Meeting, Nassau, Bahamas

April 2012 "Chromatin Responses to Double Strand Breaks, Univ. of CA, Berkeley. Berkeley, CA

April 2102 "BRCA1 Dependent DNA Repair and Tumor Suppression," University of Texas, Austin, TX.

April 2102 "Early Onset Ovarian Cancer in a Patient with Biallelic BRCA1 mutations." BRCA Symposium: From Theory to Practice. Montreal, Canada.

August 2012 "Ubiquitin Responses to DNA Double Strand Breaks." 4$^{th}$ Ubiquitin & Drug Discovery Conference in Philadelphia. Philadelphia, PA.

Sept. 2012 Interplay Between Chromatin Responses to DNA Double Strand Breaks and Transcription. 43rd Annual Meeting of the Environmental Mutagen Society. Bellevue, WA.

October 2012 Chromatin structure and DNA repair mechanism choice. New York Genome Integrity Discussion Group. New York, NY.

October 2012 Chromatin structure and DNA repair mechanism choice. Johns Hopkins Medical School. Baltimore, MD.

Dec 2012 Chromatin structure and DNA repair mechanism choice. Memorial Sloan Kettering Cancer Center. New York, NY.

Dec 2012 Chromatin structure and DNA repair mechanism choice. Genentech. San Francisco, CA

January 2013 Chromatin structure and DNA repair mechanism choice. UCSF Cancer Center. San Francisco, CA

April 2013 Chromatin structure and DNA repair. Millenium: The Takeda Oncology Company. Cambridge, MA.

May 2013 Chromatin structure and DNA repair. Mayo Clinic, Rochester, MN.

June 2013 Chromatin structure and DNA Repair. Symposium on Molecular Medicine, Drexel School of Medicine, Philadelphia, PA.

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|---|---|
| Aug, 2013 | "Novel Strategies to target deubiquitinating enzymes for inflammation" Ubiquitin & Drug Discovery Conference in Philadelphia. Philadelphia. |
| Aug, 2013 | "Chromatin Mechanisms of DNA Repair and Tumor Suppression" Peking University School of Medicine, Beijing, China |
| Nov, 2013 | "Telomere Dynamics and Cancer" New York University School of Medicine, NY, NY |
| Nov, 2013 | "Nuclear architecture and DNA repair" NIH Workshop on Chromosome Architecture and Cancer Workshop, NIH, Bethesda MD. |
| Jan, 2014 | "BRCA Function and Cancer Susceptibility" AACR Special Conference on Cancer Susceptibility and Cancer Susceptibility Syndromes. San Diego, CA |
| Jun, 2014 | Keynote Speaker, Canadian Symposium on Telomeres and Genome Integrity, Quebec City, Quebec, Canada |
| Jun, 2014 | Keynote Speaker, NCI Workshop of the Center of Excellence in Chromosome Biology, Bethesda, MD |

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Minn

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2. Grant from the Basser Research Center for BRCA

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NCI
The RAP80-BRCC36 Deubiquitinating complex in DNA repair
Role: PI

1R01 CA174904-01 (Greenberg) 04/01/2013-11/31/2018
NCI
Roles of Chromatin Modification in BRCA1 Dependent DNA Repair
Role: PI

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NIGMS
DNA Double Strand Break Chromatin Alterations and Genome Integrity
Role: PI

DOD Breast Cancer Idea Award 08/01/2012-07/31/2014
BC111503 Regulation of Metastasis and DNA Damage Resistance Pathways by Transposable Elements
Role: co-PI with Andy Minn

Harrington Discovery Institute Scholar-Innovator Award 01/01/2013-12/31/2015
Targeting DNA damage induced ubiquitin regulation for cancer chemotherapy
Role: PI

PA Breast Cancer Foundation 02/01/2013-1/31/2014
Ubiquitin regulated DNA repair and breast cancer biology
Role: PI

**V. CONCLUSION**

This funded study has established the importance of a novel mechanism of therapy resistance. Endogenous RNA from stromal cells is transferred to triple negative breast cancer cells in an exosome mediated mechanism. This activates pattern recognition receptors and interferon responsive gene expression to promote therapy resistance. These findings have been published in several high impact journals and will alter the way in

which response to therapy is viewed. Moreover, they provide new biomarkers of therapeutic response that can be examined in the context of clinical trials.